Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[7] June 2020 : 16-20

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Area Under Curve UV- Spectrophotometric Method for Determination of Cycloserine in Bulk

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ABSTRACT
The aim of this work is to establish simple, economical, and rapid spectrophotometric method for the quantification of cycloserine in Active Pharmaceutical Ingredient. Further, this study is designed to validate the developed methods as per ICH guidelines. The work was carried out to for estimation of cycloserine in bulk pharmaceutical form by utilizing area under curve (AUC) method using UV-Visible Spectrophotometry. For this purpose the wavelength range 200-400 nm was selected. 0.01N HCL was used as a solvent throughout the work. Linearity was observed in concentration range 5-25 µm/ml (r^2 = 0.9962) for the method. The developed method was found to be simple, linear, precise, accurate and sensitive which can be used for routine quality control analysis for spectrophotometric estimation of Active Pharmaceutical Ingredient.
Keywords: *Cycloserine, 0.01N HCL, UV-Visible Spectrophotometry, Area Under Curve.*

Received 21.04.2020 Revised 12.05.2020 Accepted 03.06.2020

INTRODUCTION

Cycloserine is a drug used as an antibiotic. It prevents tuberculosis bacteria from growing in your body. Cycloserine is also sometimes used to treat urinary tract and other types of infections that have not responded to other treatments. Cycloserine may also be used for purposes other than those listed medication guide. Chemically cycloserine is known asD-4-Amino-3-isoxazolidone as shown in Figure 1. Molecular formula is $C_3H_6N_2O_2$ and it is molecular weight 102.09 [1, 2]. Cycloserine which melting point on 147°C, it is white or pale yellow colour which is slightly hygroscopic in nature and degrade upon expose to humid atmosphere, So cycloserine should be kept in tightly closed container [3, 4]. A literature survey revealed a method such as UV- spectrophotometric method for the determination of cycloserine in bulk material [5, 6]. In this work two simple, economical, and rapid spectrophotometric methods have been established for the quantification of cycloserine in bulk material.
The developed methods were validated for accuracy, precision, ruggedness, and sensitivity. Accordingly, the objective of this study was to develop and validate the simple spectrophotometric method for the estimation of cycloserinehydrochloride in bulk and tablets as per ICH guidelines [7.] Drug was found to be freely soluble in 0.01N HCL which was chosen for solvent proceeding studies [8].

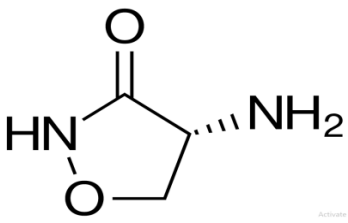


Figure 1: Chemical Structure of Cycloserine.

MATERIAL AND METHODS

Chemicals

Cycloserine was a gift sample from Wockhardt Pharmaceutical, Aurangabad. All chemicals and reagents used were of analytical reagent (AR) grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

Instrumentation

Shimadzu (Kyoto, Japan) model UV- 1800 double beam UV- Visible spectrophotometer attached with computer operated software UV probe 2.33 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Analytical balance of make Mettler Toledo (Model JL 1503- C) was used for weighing purpose.

METHOD

Experimental Work

A) To check the solubility of Cycloserine:

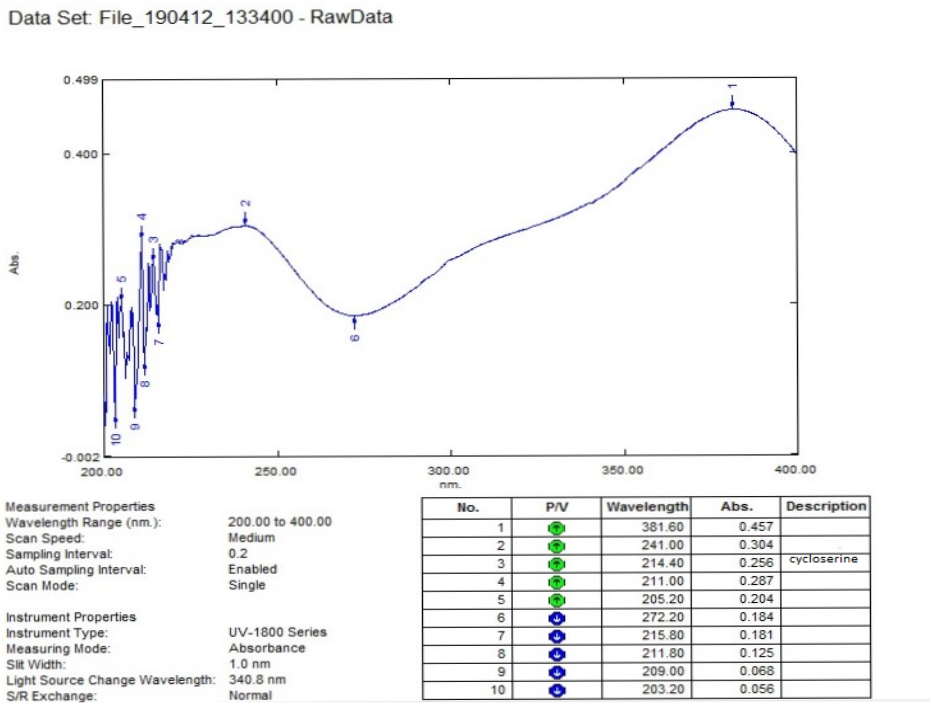
25 mg of Cycloserine was weighed and solubility of this sample was checked in 25 ml distilled water, methanol, ethanol, HCL. [6]

B) To identify the λ max of Cycloserine:

Weigh1gm of the pure drug and dissolve it in small portion of 0.01N HCL and make up the volume upto 10 ml using distilled water to give a standard stock solution of 1000µm/ml. From above solution 2.5 ml of the standard solution was withdrawn in volumetric flask and diluted to 25 ml to prepare 100ppm solution. Suitable dilutions were made with distilled water to get standard solutions of concentrations: 5, 10, 15, 20, 25 µm/ml.[9,10] Spectrum peak details are shown in Figure 2. Spectrum peak pick.

Table 1: Calibration curve of Cycloserine.

Concentration	Absorbance
5	0.187
10	0.353
15	0.546
20	0.674
25	0.831



No.	P/V	Wavelength	Abs.	Description
1	P	381.60	0.457	
2	P	241.00	0.304	
3	P	214.40	0.256	cycloserine
4	P	211.00	0.287	
5	P	205.20	0.204	
6	V	272.20	0.184	
7	V	215.80	0.181	
8	V	211.80	0.125	
9	V	209.00	0.068	
10	V	203.20	0.056	

Figure 2: Spectrum Peak Pick.

Area Under Curve Method:

In case of AUC (Area under Curve) method is applicable where there is sharp peak or broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by the entering the wavelength ranges over which area has to be calculated. This wavelength range is selected on the basis of repeated

observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration versus AUC.[11,12]

Analytical Method Development and Validation:

Linearity:

The linearity of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample. For which demonstrated that the analytical procedure is of linearity. The standard solution of Cycloserine (5, 10, 15, 20, and 25 µm/ml) was pipette out in a separated series of 10ml volumetric flask. Make up the volume with distilled water and mixed well. The absorbance maxima and area under curve for the solutions was measured at 214.40 nm and range of 200 – 400 nm for two methods respectively against distilled water as blank. Calibration Curve table of Cycloserine is shown in Table. 1.Calibration curve of Cycloserine [11, 13].

RESULTS AND DISCUSSION

A) Calibration Curve for Drug :

Absorbance maxima method:

In the Experimental conditions described, the graph obtained for the absorbance maxima for pure drug showed linear relationship Figure 3. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curve were y = 0.0322x + 0.0355(r^2 = 0.9962) at 214.40 nm for absorption maxima the range was found to be 5 - 25 µm/ml for the UV spectrophotometric analysis. Calibration Curve is shown in Table 1. Calibration Curve of Cycloserine. Calibration curve of Cycloserine is shown in Figure 3. Calibration Curve of Cycloserine.

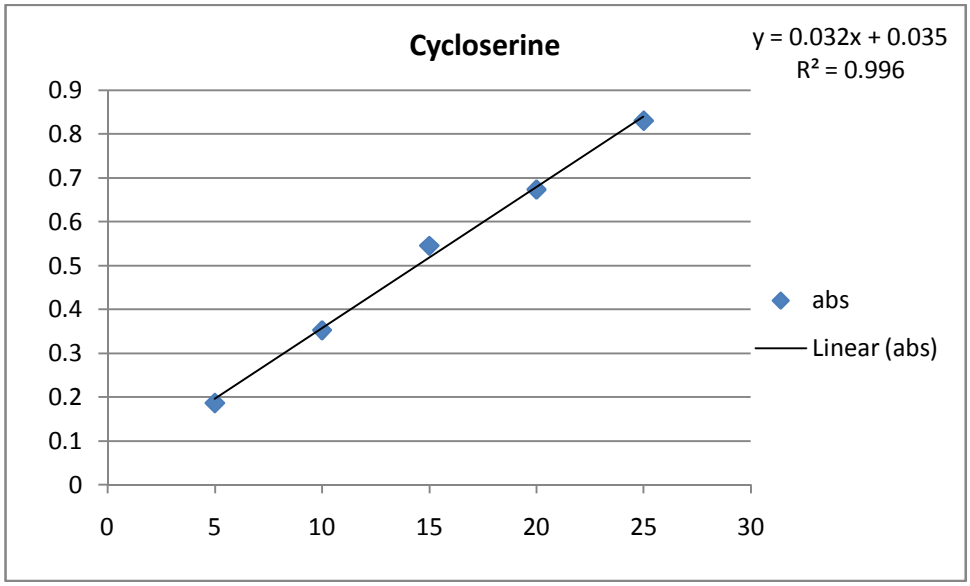


Figure 3: Calibration Curve of Cycloserine.

B) Area Under Curve Method :

In the Experimental conditions described, the graph obtained for the Area Under Curve (AUC) spectra showed linear relationship Figure 4. Regression analysis was made for the slope, intercept and correlation values. The equation is y = 0.0322x + 0.0355(r^2 = 0.9962) at 200 – 400 nm for Area Under Curve spectrophotometry analysis. The range was found to be 5 - 25 µm/ml for the Area Under Curve UV spectrophotometric analysis.

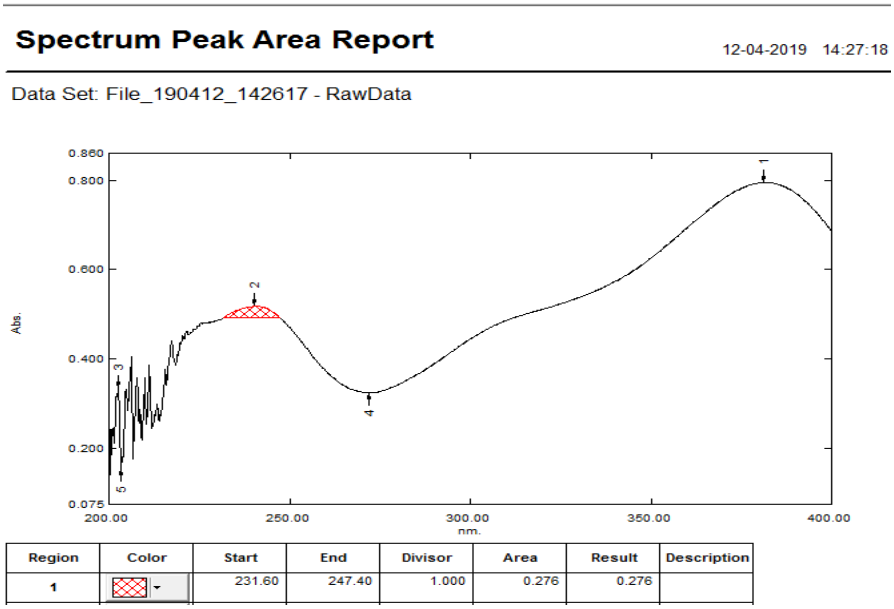


Figure 4: Area under Curve ofCycloserine.

Table 2: Area Under curve of Entacapone.

Parameter	AUC
Wavelength Range (nm)	200 – 400
Concentration Range (µm/ml)	5 – 25
Slope (m)	0.0322x
Intercept (c)	0.0355
Correlation Coefficient (r^2)	0.9962

CONCLUSION

The simple and economic UV spectrophotometric AUC methods have been developed for the determination of Cycloserine. Because of cost – effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries and successfully applied and suggested for the qualitative analysis of cycloserine in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy. The results show the UV spectrophotometric method was found to be accurate, precise and sensitive.

ACKNOWLEDGEMENT

The authors are thankful acknowledge to Head of MQA Department Vishal Institute of pharmaceutical education and research, Ale, Pune, for constant motivation and encouragement and also providing cycloserine drug as a gift sample. We would like to thank our principal Dr. Jadhav S. L. for providing us suitable environment for this work.

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CITATION OF THIS ARTICLE

Gadhave M.V., Udmale D. A., Jadhav S. L., Gaikwad D. D., Dhobale S. M. Area Under Curve UV- Spectrophotometric Method for Determination of Cycloserine in Bulk.Bull. Env. Pharmacol. Life Sci., Vol 9[7] June 2020 : 16-20